PROGRESS REPORT

PHYSICAL CHARACTERIZATION OF MAGNETIC BACTERIA AND THEIR ELECTROMAGNETIC PROPERTIES IN THE FREQUENCY RANGE 1-400 GHz

CONTRACTOR: Dr. Brian B. Schwartz
BioMagnetch Corporation

CONTRACT NO: N00014-85-C-2225

CONTRACT OFFICER: Dr. Eddie Chang
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4555 Overlook Ave., S.W.
Washington, D.C. 20375
Attn: Code 6190

PROGRESS REPORT DATE: May 14, 1986

BioMagnetech Corporation

301 East 47 Street, Suite 6A, New York, NY 10017

May 15, 1986

Ed
I'm bringing along a ½" and a ¾" tape. Thus you don't have to bring the VCR to DARPA
Brian

Dr. Ira Skurnick
DARPA-DSO
1400 Wilson Blvd.
Arlington, VA 22209

Dear Ira;

Enclosed is a progress report on the contract to BioMagnetech entitled, "Physical Characteristics of Magnetic Bacteria and Their Electromagnetic Properties in the Frequency Range 1-400 GHz". As you can read in the report, we have made significant progress in the scale-up of the magnetic bacteria and can project a cost as low as $10 per lb. of dry weight bacteria. The magnetic measurements have also gone very well and the electromagnetic measurements are well underway.

In addition to myself, Dr. R. Blakemore and Dr. R. Frankel will be coming to Washington on May 21 to participate in the review presentation. Dr. Chang asked me to prepare a budget status report updated to the review date. Attached to this letter is a graphical representation of the contract spending to date and projections. I've also included a time-line progress report based on the proposed work statement.

I have had prepared for our meeting some video tape of our fermentation experiments and hope to show portions of it at the review. We at BioMagnetech are looking forward to making our presentations at the review. If you have any special requests, please feel free to call me at (212) 750-8341.

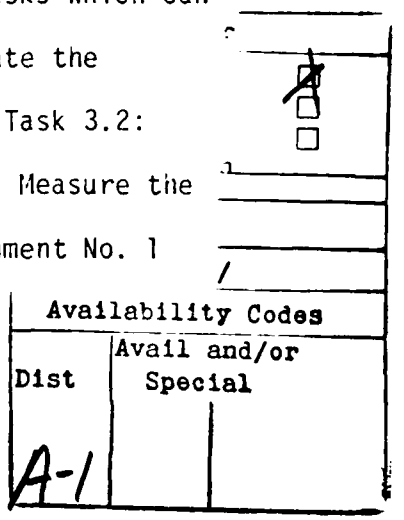
Very truly yours,

Brian B. Schwartz
President

cc Dr. Ed Chang

I. BACKGROUND

On May 15, 1985, a contract was awarded to BioMagnetech Corporation (BMC) through the Naval Research Laboratory with Dr. Eddie Chang of NRL as the contract officer and Dr. Brian Schwartz (BMC) as the contractor. The statement of work is entitled "Physical Characterization of Magnetic Bacteria and their Electromagnetic Properties in the Frequency Range of 1-400 GHz." The contract was divided up into three main tasks which can be grouped under three main headings: Task 3.1: Investigate the scale-up of culturing and harvesting of magnetic bacteria; Task 3.2: Study basic properties of magnetic bacteria; and Task 3.3: Measure the electromagnetic properties of magnetic bacteria (see Attachment No. 1 from the original contract).



II. OVERVIEW

The progress to date has been excellent. The first main question which had to be answered was, "Can the bacteria be grown in sufficient quantities by standard fermentation techniques at a reasonable cost?" We first investigated which, if any, of the chemical nutrients in the culture medium might be limiting the growth of the magnetic bacteria. Next, we ran scale-up experiments in a 10 liter fermentor/chemostat operated as a semi-continuous batch culture. By obtaining a proper balance with respect to nitrogen and carbon, we have already increased the yield by a factor of 10. In addition, research on the components and cost of the culture medium allow us to project a moderate cost of less than $100 per lb. of dry bacteria, with a possible final projection as little as $10 per lb.! Significant research remains to be done,

especially in improving the magnetic yield per bacterial cells before one can confidently proceed to large-scale manufacture.

In order to interpret the electromagnetic properties of magnetic bacteria, a good understanding of the basic magnetic properties of the bacteria must be studied. We have made magnetic measurements on samples of freeze-dried cells. The measurement techniques included a vibrating sample magnetometer and a rotating sample magnetometer. The measurements made include: DC hysteresis loops to $\pm$600 Oe, and 1200 Oe, and a comparison with theory, components of the normal magnetization, the anisotropy field, the hysteresis and initial magnetization of a spinning sample, and the hysteresis loop for fixed cells in water frozen in a field of 900 Oe. From these measurements we obtain the saturation magnetization as 0.60 emu/gm, the coercive force as 160 Oe, and the ratio of the remanent magnetization to the saturation magnetization equal to 0.43. These measurements indicate that in the freeze-dried sample, the iron is in the form of magnetic Fe_3O_4 with a 1% dry weight of the cells. Measurements with cells dried on mylar samples are in progress. In addition, magnetite bacteria are being grown with increased magnetosomes per cell so as to increase the magnetic response. The magnetic behavior of these cells will be investigated in various configurations.

Initial experiments to measure the electromagnetic properties of magnetic bacteria are underway. The bacteria cells have been prepared in special holders dispersed in a background mixture of lanolin, vaseline, and paraffin. The initial experiments will answer questions on the dielectric permittivity and IR absorption. The scale-up fermentation should provide sufficient sample volumes to conduct precise measurements. Various configurations using aligned cells will be investigated.

In Figure 1 we present an overview of progress to date on all three subtasks. Some of the exciting results to date include excellent developments on scale-up and the lowering of cost, and significant understanding of the basic magnetic properties. (Note: we have also initiated, in collaboration with Stanford Research Institute, a feasibility study of genetic engineering of the genes controlling magnetite production in magnetic bacteria.) The electromagnetic measurements are in progress and we expect to have absorption data shortly. In Section III we detail the progress on Task 3.1, scale-up. In Section IV we present the measurement on the basic properties, and in Section V we discuss the measurements initiated on the electromagnetic properties. This is followed by a list of figure captions, figures, and references.

III. TASK 3.1: SCALE-UP OF MAGNETIC BACTERIA

A. EVALUATION OF CELL CULTURE MEDIUM:

Prior to experimental work directed at scale-up, analysis was made of the culture medium used to grow the magnetic spirillum Aquaspiriullum magnetotacticum. This was considered important to evaluate which, if any, medium constituent might be limiting for growth. Related work by R.P. Blakemore had shown that magnetic spirilla do not produce toxic metabolic products which might limit their own growth in this medium because they had been cultured successfully at very high cell densities as colonies in solidified medium of this type.

Prior to the inception of this contact (1), the medium had the composition shown in Table 1. As a preliminary, the maximum cell growth

TABLE 1. Magnetic spirillum growth medium (MSGM).

Component	Added amount per liter
Tartaric acid	0.37 g
Succinic acid	0.37 g
Sodium acetate	0.05 g
Sodium nitrate	0.17 g
Monopotassium phosphate	0.69 g
Sodium thioglycollate *)	0.06 g *)
Resazurin (stock 1 g/L)	8 drops
Ferric quinate (0.01 M) **)	2 ml **)
Minerals ***)	5 ml ***)
Vitamins ****)	0.5 ml ****)
Distilled water	1 liter

*) Alternatively, 0.03 g ascorbic acid
**) Stock solution of 2.7 g/L FeC13 and 1.9 g/L Quinic acid
***) Mineral medium (pH 6.5 w/KOH) containing per liter:

Nitrilotriacetic acid	1.5 g
MgSO4.7H2O	3.0 g
MnSO4.H2O	0.5 g
FeSO4.7H2O	0.1 g
CoSO4	0.1 g
CaC12	0.1 g
ZnSO4	0.1 g
CuSO4.5H2O	0.01 g
AlK(SO4)2	0.01 g
H3BO3 (boric acid)	0.01 g
Sodium molybdate	0.4 g
Water	1 liter

****) Vitamin medium containing per liter:

Biotin	20 mg
Folic acid	20 mg
B-6 (pyridoxine HCl)	100 mg
B-1 (thiamine HCl)	50 mg
B-2 (riboflavin)	50 mg
Niacin	50 mg
Panthotenic acid (DL Ca-salt)	50 mg
B-12	1 mg
PABA	50 mg
Lipoic acid	50 mg

TABLE 2. Estimated maximum cell concentrations based upon the elemental composition of the growth medium.

Element	In medium; mg/L	In dry cells; % (by weight)	Maximum cell growth; g/L (dry weight)
Nitrogen	28	7-14	0.2-0.4
Potassium	194	0.5-1	19.4-38.8
Phosphorus	154	1-3	5.1-15.4
Sulfur	3.2 *)	0.5-1	0.3-0.6 *)
Magnesium	1.5	0.5-1	0.15-0.3
Iron	2	0.3-2	0.1-0.7 **)
Oxygen	60 ***)	---	0.03-0.06 ***)
Carbon	280	--- aerobic	0.2-0.3 ****)
Carbon	280	--- anaerobic	0.04-0.1 *****)

*) Assuming no thioglycollate in medium (using ascorbate as reducing agent)

**) Magnetic vs. nonmagnetic cells (iron contents of cells from Table 1 in Blakemore et al. 1979 publication)

***) Assuming aerobic growth; 0.5-1.0 g cell/g oxygen -- The oxygen concentration is here calculated assuming 1% oxygen in headspace (giving 30 mg/L equivalent oxygen concentration) plus oxygen available from nitrate to nitrite reduction.

****) Aerobic growth; 0.3-0.4 g cell/g tartaric acid equiv.

*****) Anaerobic growth; 0.05-0.15 g cell/g tartaric or equiv.

allowed by this medium was calculated based upon typical elemental composition of microbial cells. These calculations appear in Table 2. The results suggested that increased growth yields would result by increasing the concentrations, in descending order of (1) carbon source, (2) oxygen (3) iron, (4) magnesium, (5) nitrogen, and (6) sulfur. Previous studies had shown, however, that increased oxygen would stimulate the yield of cells cultured with NH_3 as the N source but would also lead to a high percentage of non-magnetic (non-magnetite containing) cells in the population. Thus, any attempt to increase cell yields for scale-up must include evaluation of the magnetic state of the cells as a function of the medium they were cultured in and growth conditions.

B. MASS CULTURE OF CELLS:

The magnetic spirillum was mass cultivated in a small (10 L) fermentor/chemostat operated as a semi-continuous feed batch culture in order to evaluate proposed changes in its culture medium as discussed above. The culture apparatus was sterilizable in-place and equipped to monitor dissolved oxygen (2) (via galvanic electrodes) and pH control. In-line gas analysis via a mass spectrometer was carried out for oxygen and carbon dioxide in the fermentor off-gas.

Initially unsuccessful efforts to establish spirillum growth in this apparatus were evaluated carefully and subsequent modifications providing for more protection against oxygen toxicity were incorporated. Eventually a strategy of slow intermittent nutrient feed, use of stringent precautions for maintaining dissolved oxygen at or below 0.1% of saturation (but not completely anaerobic), together with use of a heavy inoculum, resulted in reproducible, successful growth initiation.

Subsequent efforts were directed at increasing growth yields of the bacteria.

C. ENHANCEMENT OF CELL YIELD:

By intermittent feed of nutrients "balanced" with respect to nitrogen and carbon, it has been possible to increase the culture yield of A. magnetotacticum by a factor of 10 over that obtained prior to this contract. Figure 2 shows the growth of a culture of this organism in the apparatus described. Growth is linear rather than exponential. However, final cell yields are currently approximately 5×10^9 cells/ml and are expected to increase, perhaps by another factor of 2 to 5 before this phase of the contract is concluded. This initial success has been accomplished through manual adjustments (e.g., manual addition of iron or oxygen) in response to cell growth or by automated delivery in the case of carbon and nitrogen (via pumps operating in response to shifts in culture pH values). All of these operations can be automated in scaled-up procedures by means of computer interfacing.

In this work, consideration has been given to the costs of medium components and materials. It is fortunate that high culture yields of this organism (comparable to those of Escherichia coli) are now possible, but in a simple mineral medium containing tartaric acid as the carbon and energy source. Tartrate is a relatively cheap commodities chemical well suited to mass culture of micro-organisms in industrial scale operations. As a consequence of this work it is already possible to project (based upon current cell yields) a moderate cost of less than $100., possibly as little as $10 per lb. dry bacteria.

How?

D. WORK IN PROGRESS:

While the bacteria produced in these improved laboratory scale mass cultures appeared to be "reasonably" magnetic, several non-magnetic (spirillum) cells were also present. These results suggest that additional work should be carried out to improve the magnetite yield per bacterial cell. In parallel studies, we have determined that the culture medium iron content influences directly the number of magnetosomes per cell over the range 1-40 μm iron supplied as ferric quinate. Likewise, the cell magnetosome content bears direct relation to the culture-dissolved oxygen.

From what we learned there remains some questions to be answered. The main remaining question is, what is controlling the magnetic contents of the cells? It is obviously necessary to get enough iron to the cells, but it is equally clear that this criterion is not sufficient. The cells "often" become (irreversibly?) non-magnetic through multiple transfer. If some selection exists against magnetic bacteria, this problem could increase with increasing fermentation scale. Listed below are some questions that we are studying before we can proceed to large-scale manufacture.

(1) Which form of iron (Fe++ vs Fe+++, chelated vs free iron) is preferentially assimilated by the bacteria?

(2) Can the generation of the desired iron-ion form become rate limiting for magnetite production (particularly at high cell densities)?

if magnetite confers ~~survival~~ survival pts., then growth in fermenter multiplies this Vs mag. field & oxygen gradient

(3) Can the number of cell divisions (i.e., inoculum size) affect the fraction magnetic cells of the population (genetic selection)?

(4) Will differences in nitrogen source (NH_3 vs HNO_3) affect the magnetic contents of the cells if the oxygen and iron availability otherwise is similar and sufficient (it will be easier obtaining high cell yields and growth rates using NH_3)?

(5) Is the magnetite production growth or non-growth associated (i.e., will it depend on the cell doubling rate)?

Many of these questions will be answered using batch cultures, since the experiments will be designed such that high cell concentrations are not required to obtain the answers. However, these batch cultures will be operated somewhat differently from the methods used in the past in order to eliminate secondary effects (such as oxygen depletion, pH problems, nutrient limitations, etc.).

IV. TASK 3.2: BASIC PROPERTIES OF MAGNETIC BACTERIA

A. MAGNETIC MEASUREMENTS:

Initial measurements were made on samples of freeze-dried cells which were harvested previously. The sample consisted of 56.4 milligrams of cell powder compressed into a 5mm disk. The D.C.hysteresis loop was measured using a vibrating sample magnetometer after saturating the sample magnetically at 90^0 to the measuring axis. Additional measurements were made with a rotating sample magnetometer.

Figures 3A and 4 show the initial magnetization and hysteresis curves measured to ±600 Oe and ±1200 Oe, respectively. The primary quantities determined by the hysteresis loop are the coercive force, H_C, and the ratio of the remanent magnetization, M_R, to the saturation magnetization, M_S. The saturation magnetization σ_S was measured in an applied field of 6 kOe. For this sample, σ_S = 0.60 emu/gm, H_C = 160 kOe, and M_R/M_S = 0.43.

The value of σ_S is equivalent to an Fe_3O_4 content of about 1% dry weight of the cells. The shape of the hysteresis loop is consistent with the Stoner-Wohlfarth (SW) model (3) of coherent rotation. A theoretical hysteresis loop calculated from the SW model for a random array of non-interacting, randomly oriented magnetic dipoles of single-domain character with uniaxial anisotropy, H_A, is shown in Figure 3B. H_A was determined as described below.

The distribution of anisotropy energies in the sample was determined by measuring the component of magnetization normal to the magnetizing field H after magnetizing the sample to saturation, reducing the field to zero, and rotating the sample by 6 degrees (4,5). The data for successive field cycles of increasing magnitude up to 700 Oe are shown in Figure 5A. From these data, the anisotropy field distribution was calculated and plotted in Figure 5B. The mode of the anisotropy distribution H_A = 440 Oe.

This value was checked by measruing the initial susceptibility of a spinning sample with a Hall probe technique, as shown in Figure 6. The result is H_A (average) = 476 Oe, in good agreement with the other method. This value of H_A was used with the SW model to calculate the theoretical hysteresis loop in Figure 3B.

Measurements were also made on fresh samples of magnetic bacteria grown in batch culture that were fixed in 1% gluteraldehyde following growth and harvesting. A portion of this batch was subsequently freeze-dried for electromagnetic measurements. Another portion was kept in dense supsension.

Measurements of the orientation of individual cells in the suspension at 300 K was made with a SQUID magnetometer. The magnetization approaches saturation for fields above 10 Oe, as expected for an array of permanent magnetic dipoles with moments of the order of 10^{-12} emu (6). We originally hoped to measure the initial susceptibility between 0 and 5 Oe, but trapped flux in the superconducting magnet in the system below 5 Oe made the measurements unreliable.

Measurements were also made on the suspension of cells after freezing in an applied field in the magnetometer. By following the moment with temperature during the cooling process, it was determined that the suspension froze below 265 K. The freezing process itself was found to disrupt the alignment of the cells to an extent that depended on the applied field. At 100 Oe, the orientation decreased to about 60% of the saturation value. At 900 Oe, the orientation decreased to 95% of the saturation value. Using this sample the complete hysteresis loop was determined as shown in Figure 7. From these data, H_C = 285 and M_R/M_S = 0.67. This increase in M_R/M_S over the randomly oriented samples (theoretical value for M_R/M_S = 0.5) means that it should be possible to obtain well-ordered arrays of magnetic cells on substrates. Measurements with cells dried down on mylar substrates in external magnetic fields are currently in process. Alignment of the sample on the substrate will cause deviations of the M_R/M_S ratio from 0.5.

B. ELECTRON MICROSCOPY:

Research on the nature of the magnetosomes, their morphology, and crystal structure have been carried out. In Figure 8 we show an electron microscope picture of the membrane of the magnetosome.

V. TASK 3.3: ELECTROMAGNETIC PROPERTIES OF MAGNETIC BACTERIA

Samples have been prepared for electromagnetic measurements. Magnetic bacterial cells were freeze-dried following gluteraldehyde fixation. About 1 gram of freeze-dried cells was thoroughly dispersed in a special mixture consisting of lanolin, vaseline, and paraffin, 1:1:1 by weight. This mixture forms a low melting point (50°C), low EM absorbing fluid. Sample holders were made by machining 3-inch diameter dishes out of polyethylene, another low absorption material. In initial experiments, an empty dish, a dish filled with the suspension fluid, and a dish filled with the cells in the suspension fluid will be measured in order to determine the absorption characteristics of the cells themselves.

Additional measurements will be tried with freeze-dried cells suspended in liquid hexane.

Ultimately, it is hoped that suspensions with aligned cells can be prepared by allowing the suspension mixture to solidify in external magnetic fields.

REFERENCES

1. R.P. Blakemore, D. Maratea, and R.S. Wolfe, J. of Bact. 140, 720 (1979).

2. R.P. Blakemore, K.A. Short, D.A. Bazylinski, C. Rovenblatt, and R.B. Frankel, Geomicrobio. J. 4, 53 (1985).

3. E.C. Stoner and W.P. Wohlfarth, Trans. Roy. Soc. (Lond.) A240, 599 (1948).

4. P.J. Flanders and S. Shtrikman, J. Appl. Phys. 33, 216 (1962).

5. P.J.Flanders, J. Appl. Phys. 53, 2567 (1982).

6. R.B. Frankel and R.P. Blakemore, J. Magn. Magn. Mater. 15-18, 1562 (1980).

Statement of Work

Physical Characterization of Magnetic Bacteria and their Electromagnetic Properties in the Frequency Range of 1-400 GHz

1.0 Scope

This contract is for the study and characterization of magnetotactic bacteria as a possible novel class of radar absorbing material (RAM).

2.0 References

BioMagnetech Corporation Unsolicited Proposal, "The Physical Characteristics of Magnetic Bacteria and Their Electromagnetic Properties in the Frequency Range of 1-400 GHz".

3.0 Requirements

3.0.1 For the period of the contract, the contractor shall perform the following tasks:

3.1 Investigate the scale-up of culturing and harvesting of magnetic bacteria:

3.1.1 Investigate methods for scaling up the culturing of magnetic bacteria.

3.1.2 Evaluate centrifugation and magnetic separation methods for harvesting.

3.1.3 Initiate evaluations on integrating scaled-up fermentation and harvesting methods into an automated process.

3.2 Study basic properties of magnetic bacteria:

3.2.1 Characterize the magnetic properties, such as the saturation magnetization, the remanent magnetization, the coercive field, and the switching field distribution of the magnetic bacteria.

3.2.2 Extract magnetosomes and break chains into various lengths then make magnetic measurements on them.

3.2.3 Perform electron microscopy characterizations on the magnetic bacteria and magnetosomes.

3.2.4 Investigate various matrix configurations of different lengths and packaging of magnetosomes with and without membranes to increase the volume concentration of magnetic material.

3.2.5 Investigate the reactivity of magnetosome membranes.

3.2.6 Assess the possibility of altering the morphology, composition, and crystalline structure of magnetosomes through genetic manipulation, species variation, and alteration of food intake.

3.3 Measure the electromagnetic properties of magnetic bacteria

3.3.1 Perform measurements on the complex dielectric permittivity and magnetic permeability of magnetic bacteria from 1-400 GHz.

3.3.2 Make IR absorption measurements on the magnetosome membranes.

4.0 Deliverables

4.1 Progress Reports

4.1.0 Progress reviews will be undertaken in oral reviews with the COTR. These reviews shall be as deemed necessary by the COTR in order to ensure timely integration of contract results into Department of Navy program plans.

4.2 Technical Reports

4.2.0 Contractor findings shall be in writing and shall include a comprehensive interpretation of the research performed on this contract. The report shall be submitted within sixty days of the completion of the contract's tasks as described in Sections 3.1, 3.2, and 3.3 herein.

4.3 Cost Reports

4.3.0 The contractor shall submit quarterly cost reports withing fifteen days of the close of each quarterly reporting period to NRL Code 6300.1. This cost report shall itemize, for the period, all charges to the contract. This report shall be submitted instantly should facts surface which could negatively affect completion of the work within the time and cost extimates of this contract. This special report shall include a discussion of those issues affecting such conclusions.

-3-

FIGURE CAPTIONS

Figure 1: A timeline progress report on Tasks 3.1, 3.2, and 3.3.

Figure 2: Growth and cell density magnetic bacteria in a 10 liter fermentor/chemostat as a function of time and additives to the medium.

Figure 3: (A) DC hysteresis loop for freeze-dried magnetic bacteria samples to ±600 Oe. (B) Hysteresis loop computed from the SW theory for a distribution of anisotropies shown in Figure 3A. The calculation gives H_C = 135 Oe and M_R/M_S = 0.5

Figure 4: DC hysteresis loop to ± 1200 Oe.

Figure 5: (A) Component of magnetization normal to H as described in the text. (B) Anisotropy field (H_A) distribution from Figure 3A.

Figure 6: Hysteresis loop of stationary sample and initial magnetization curve of a spinning sample for determination of average anisotropy field.

Figure 7: Hysteresis loop to ± 900 Oe for a suspension of fixed cells in water frozen at 900 Oe.

Figure 8: Electron microscope picture of the membrane.

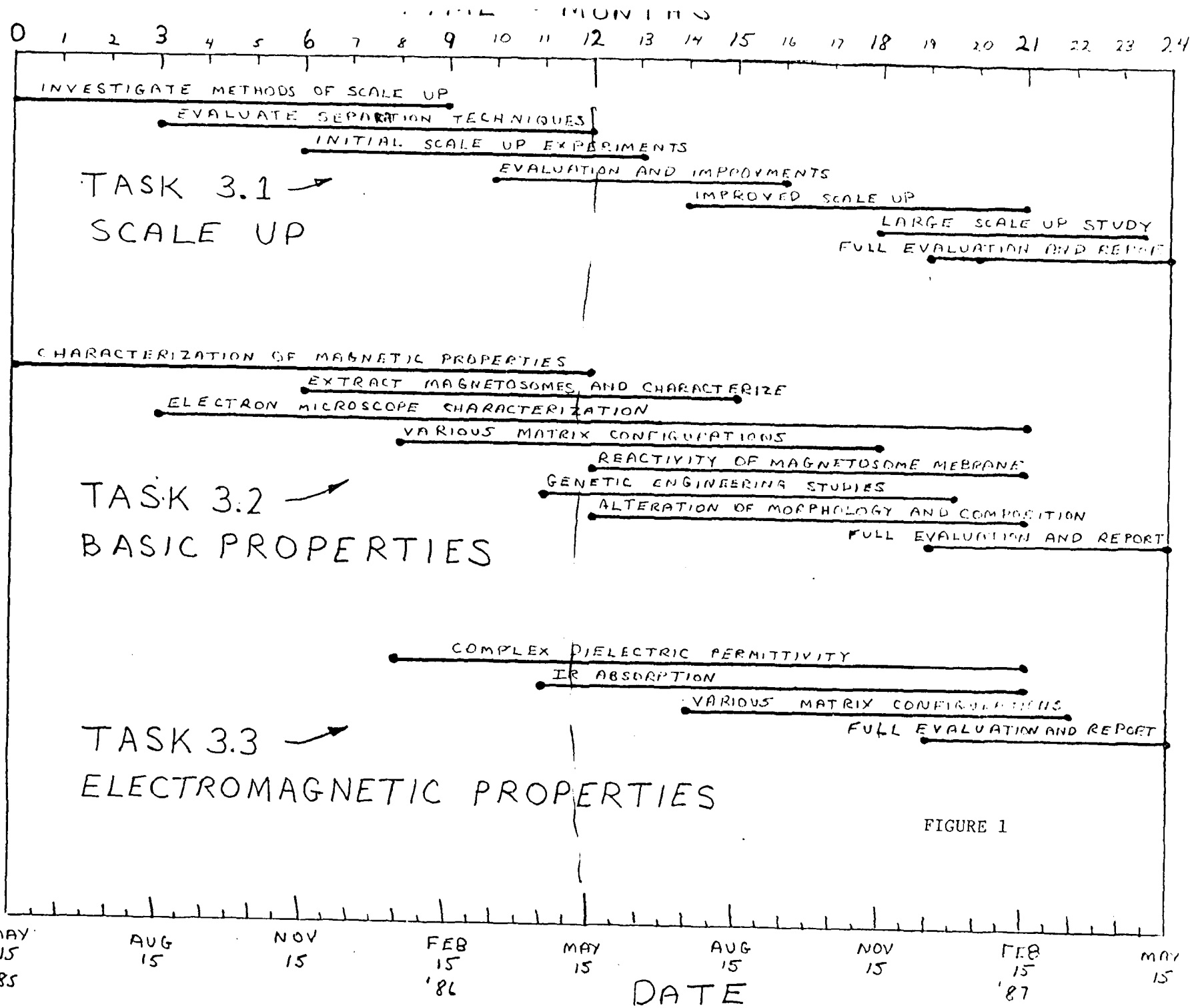

MONTHS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
INVESTIGATE METHODS OF SCALE UP
EVALUATE SEPARATION TECHNIQUES
INITIAL SCALE UP EXPERIMENTS
EVALUATION AND IMPROVMENTS
IMPROVED SCALE UP
LARGE SCALE UP STUDY
FULL EVALUATION AND REPORT
TASK 3.1
SCALE UP
CHARACTERIZATION OF MAGNETIC PROPERTIES
EXTRACT MAGNETOSOMES AND CHARACTERIZE
ELECTRON MICROSCOPE CHARACTERIZATION
VARIOUS MATRIX CONFIGURATIONS
REACTIVITY OF MAGNETOSOME MEBRANE
GENETIC ENGINEERING STUDIES
ALTERATION OF MORPHOLOGY AND COMPOSITION
FULL EVALUATION AND REPORT
TASK 3.2
BASIC PROPERTIES
COMPLEX DIELECTRIC PERMITTIVITY
IR ABSORPTION
VARIOUS MATRIX CONFIGURATIONS
FULL EVALUATION AND REPORT
TASK 3.3
ELECTROMAGNETIC PROPERTIES
MAY 15 '85
AUG 15
NOV 15
FEB 15 '86
MAY 15
AUG 15
NOV 15
FEB 15 '87
MAY 15
DATE

FIGURE 1

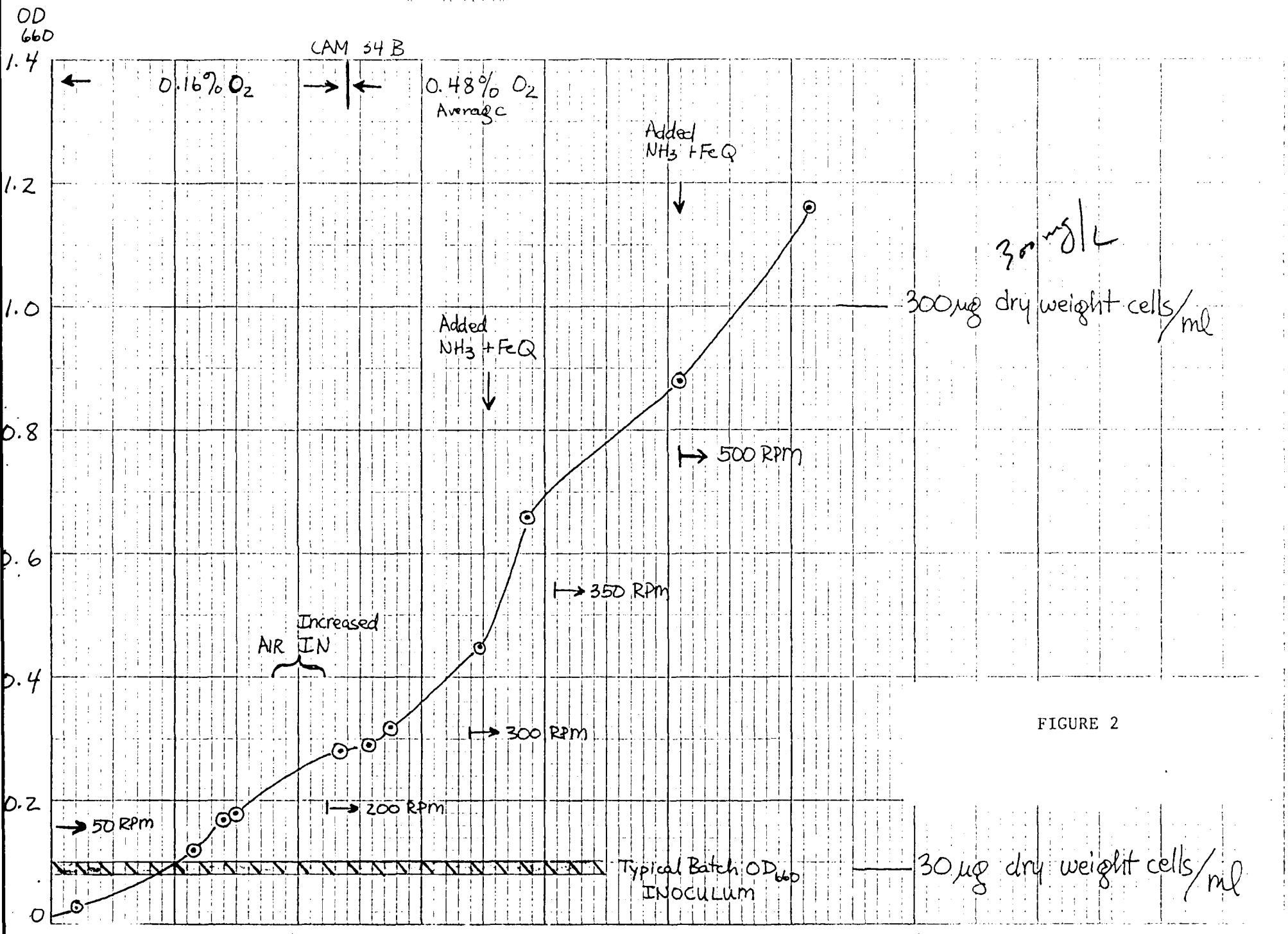

OD 660
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
CAM 54 B
0.16% O_2
0.48% O_2
Average
Added NH_3 + FeQ
Added NH_3 + FeQ
300 mg/L
300 µg dry weight cells/ml
500 RPM
350 RPM
300 RPM
200 RPM
50 RPM
Increased AIR IN
Typical Batch OD_{660} INOCULUM
30 µg dry weight cells/ml

FIGURE 2

)-A

46 1512

Bacteria 2-1-85 VSM DC hysteresis loop (±6000 oe)

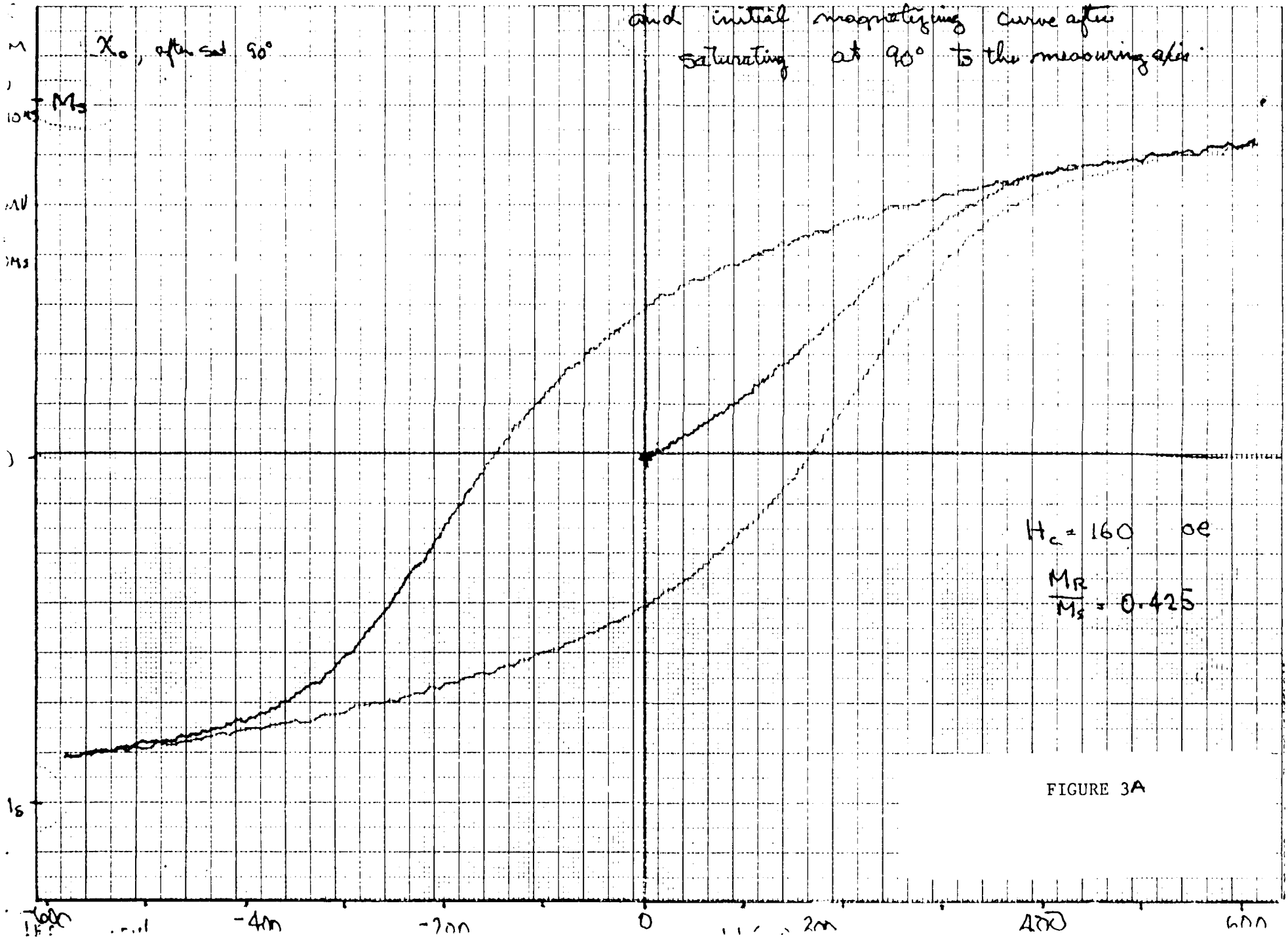


FIGURE 3A

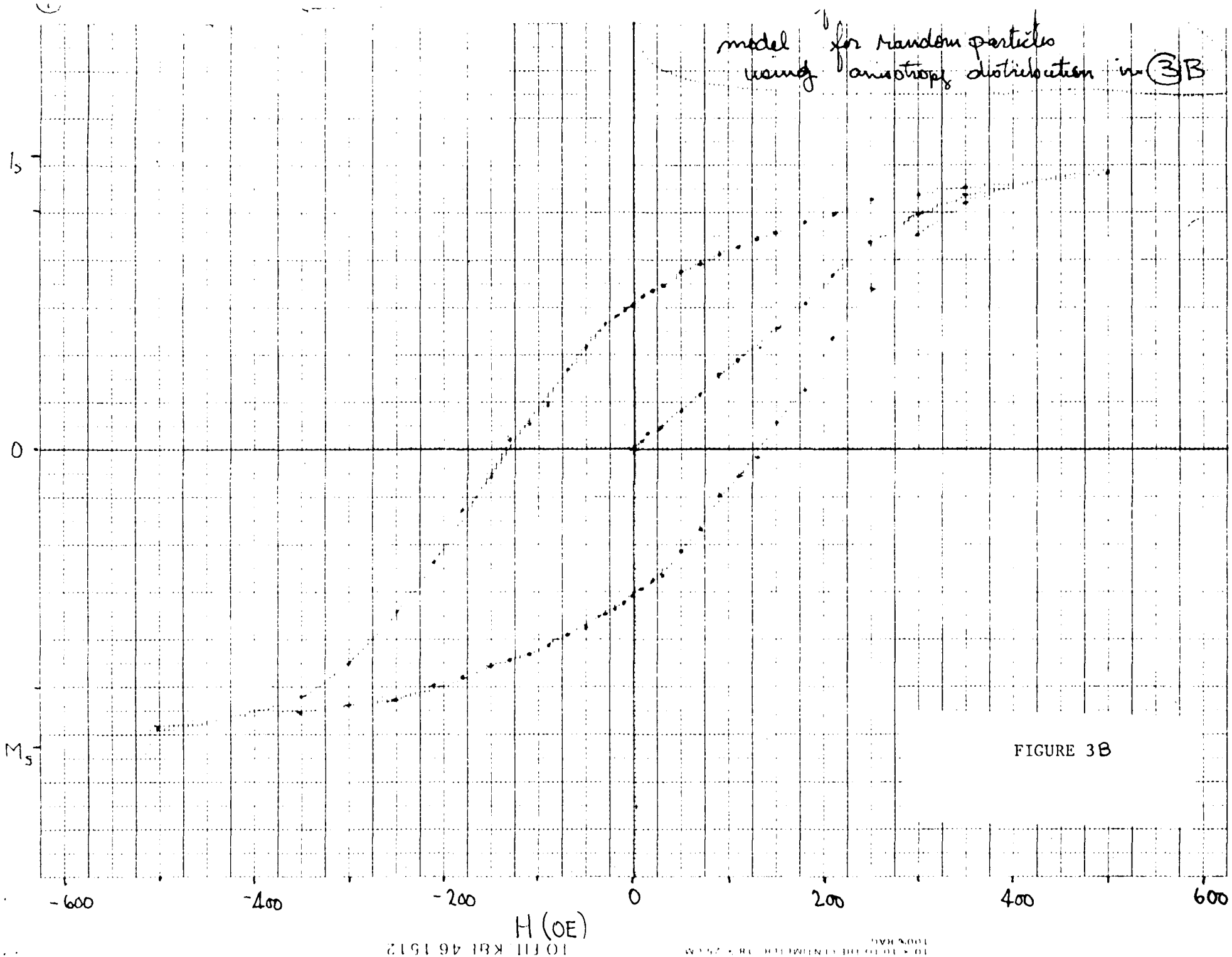

model for random particles
having anisotropy distribution in (3B)
I_S
0
M_S
-600
-400
-200
0
200
400
600
H (OE)

FIGURE 3B

BARIUM

D.C. hysteresis loop (H ± 1200 Oe) and initial magnetization curve after saturating at 90° to measuring axis.

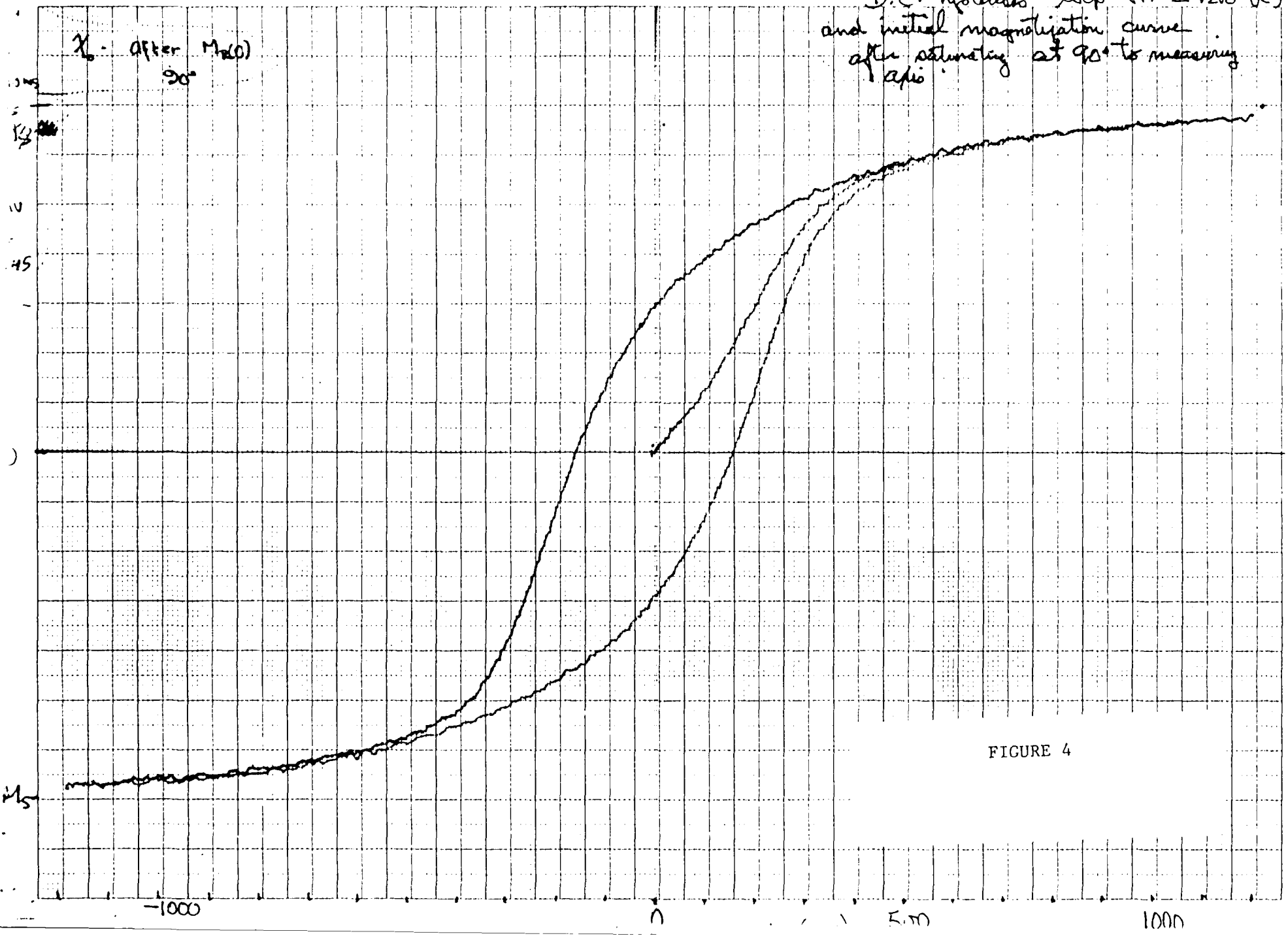


FIGURE 4

(3) A Finding Anisotropy distribution VSM 1-29-85

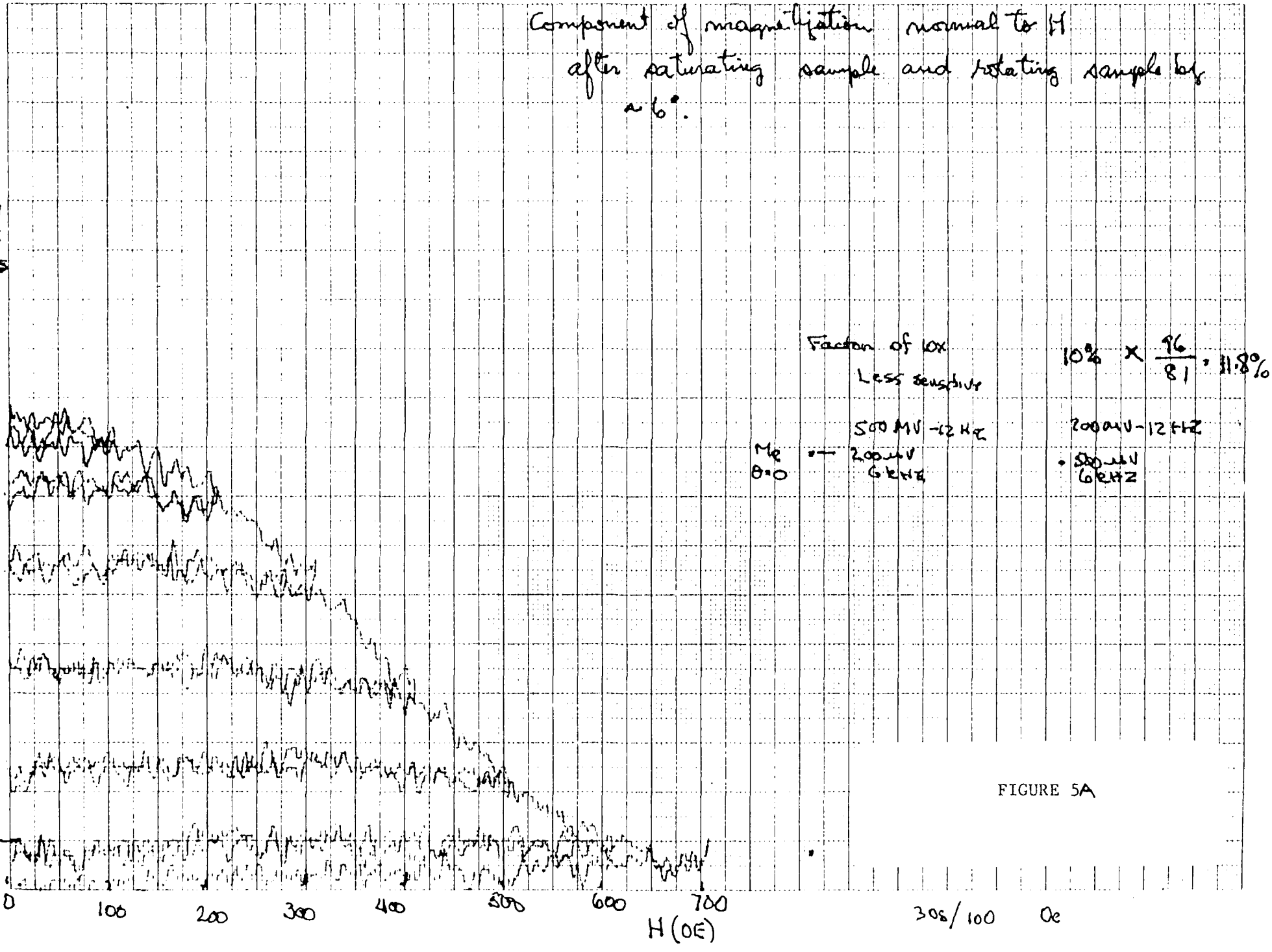


FIGURE 5A

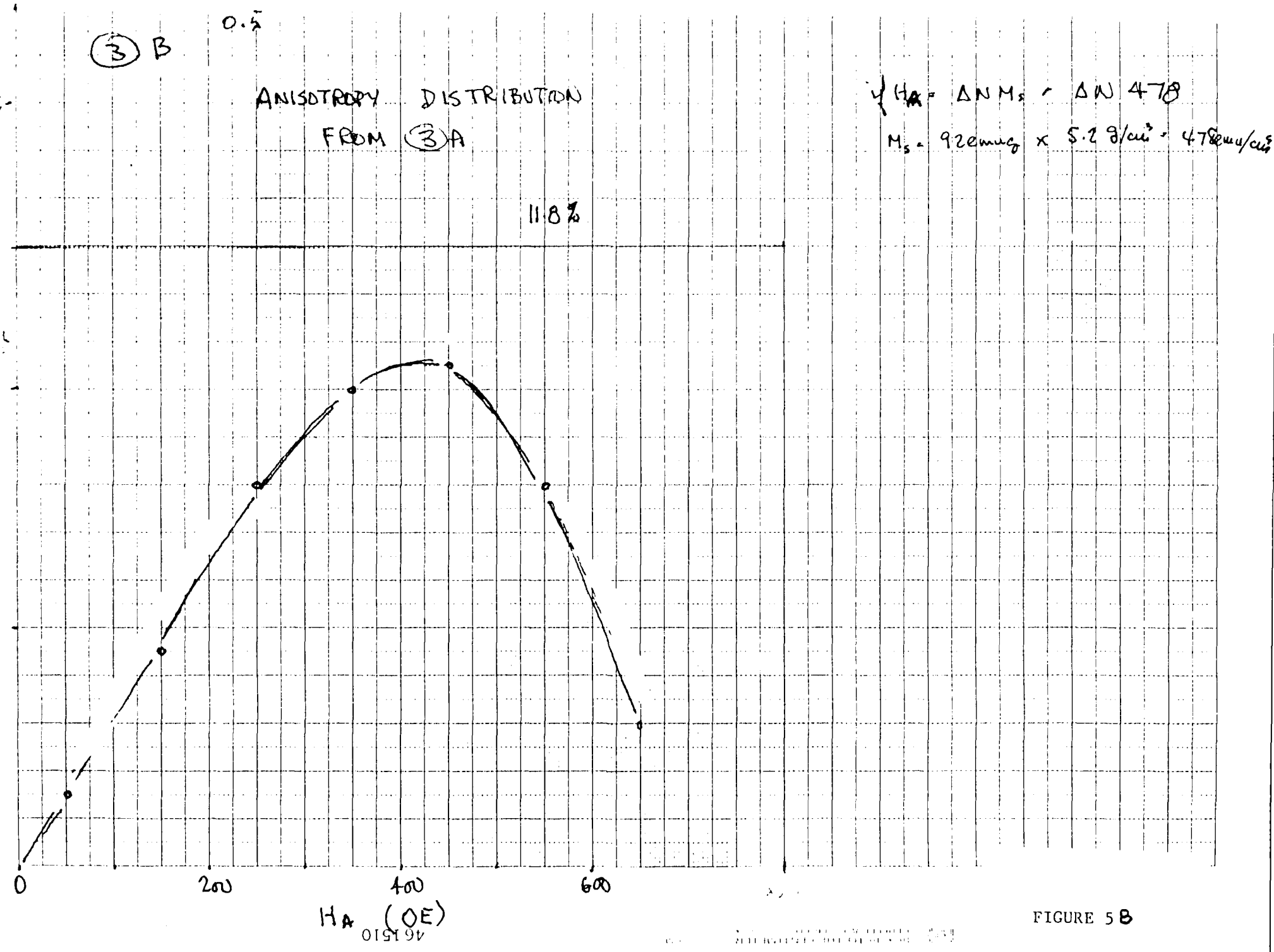

(3) B
0.5
ANISOTROPY DISTRIBUTION
FROM (3) A
11.8%
$H_A = \Delta N M_s - \Delta N 478$
$M_s = 92 emu/g \times 5.2 g/cm^3 = 478 emu/cm^3$
VOLUME DISTRIBUTION (ARBITRARY)
0
200
400
600
H_A (OE)

FIGURE 5B

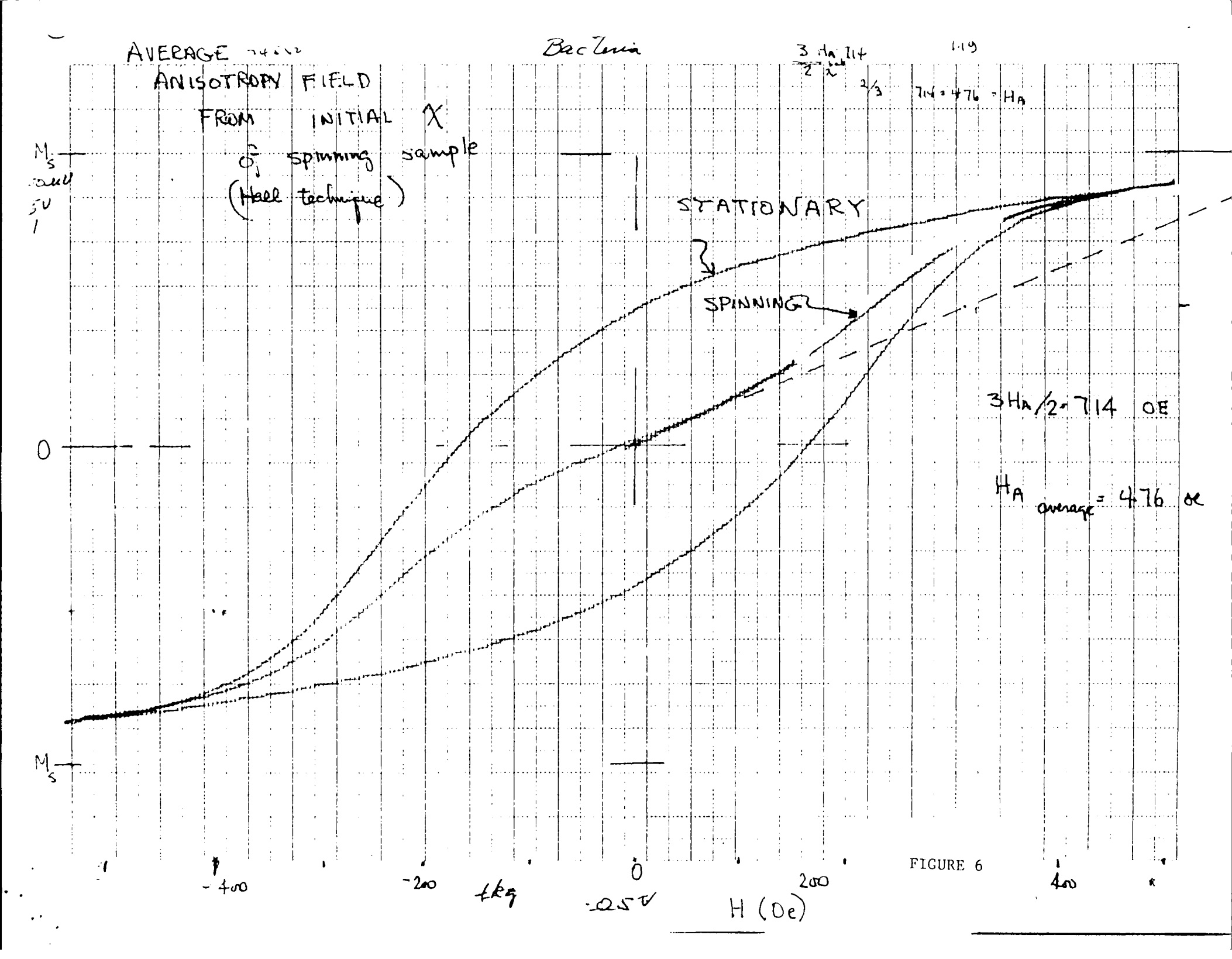

AVERAGE
ANISOTROPY FIELD
FROM INITIAL χ
σ⊥ spinning sample
(Hall technique)
STATIONARY
SPINNING
M_s
0
M_s
$3H_A/2 = 714$ Oe
H_A average = 476 Oe
-400
-200
0
200
400
H (Oe)

FIGURE 6

FIGURE 7

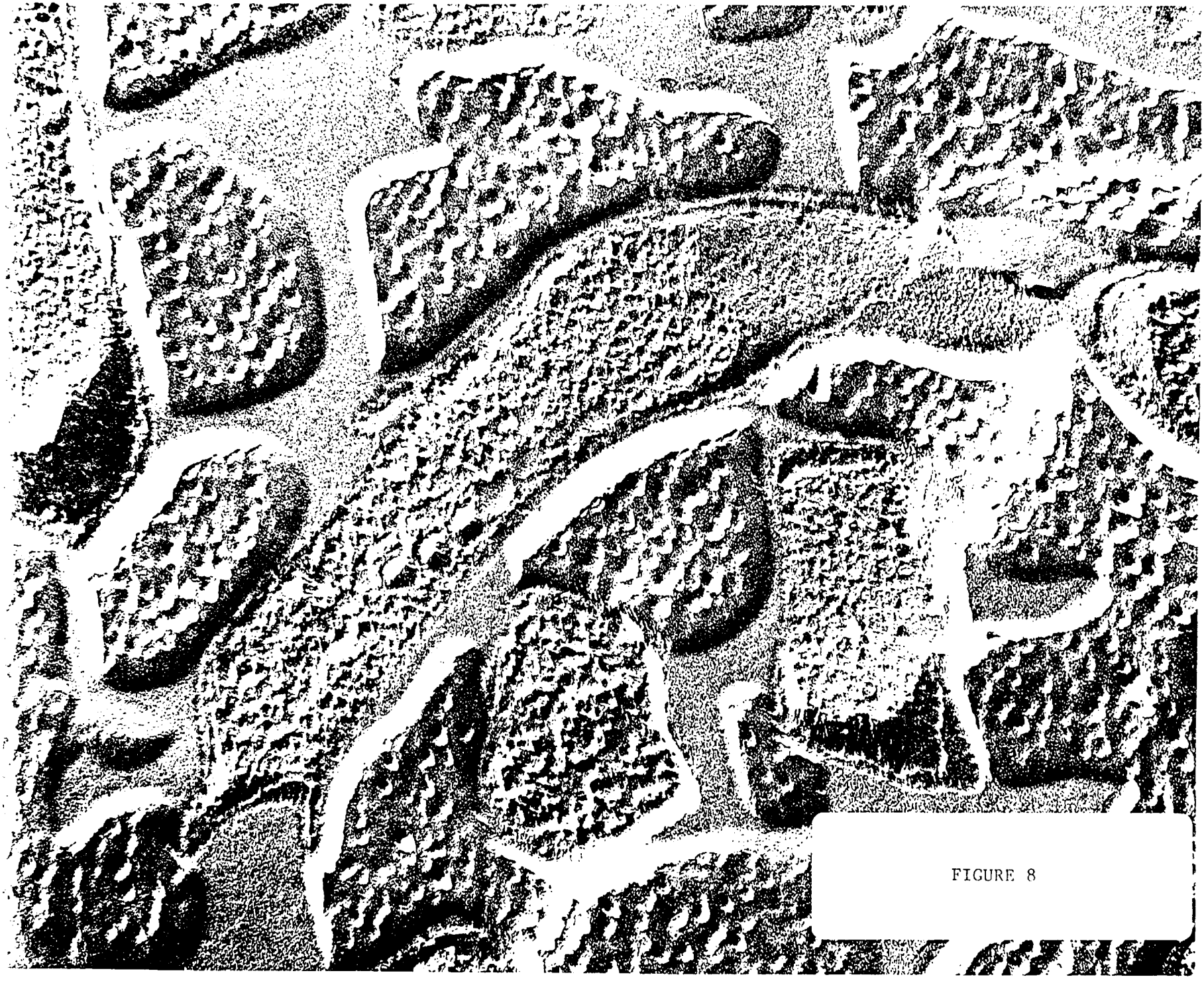
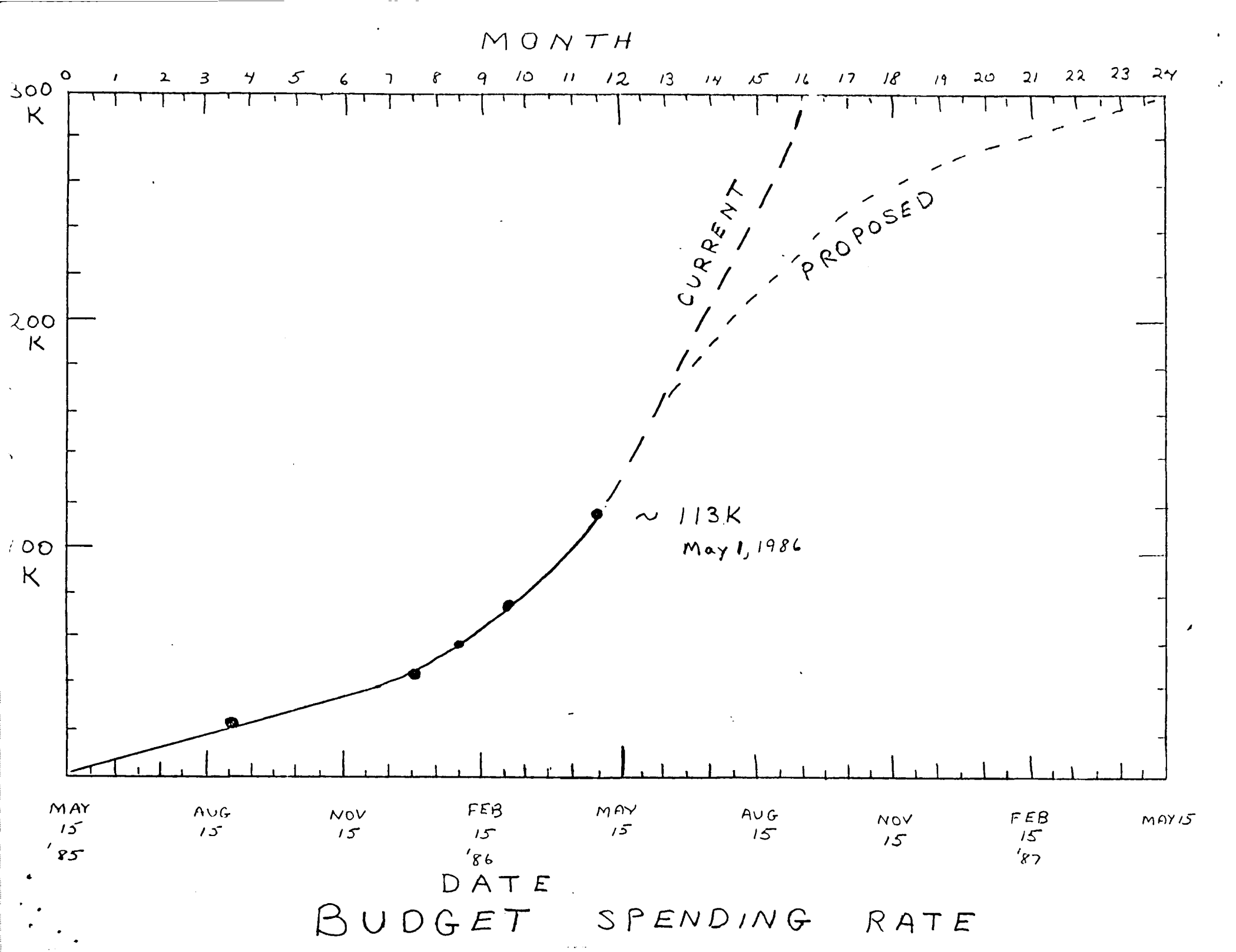
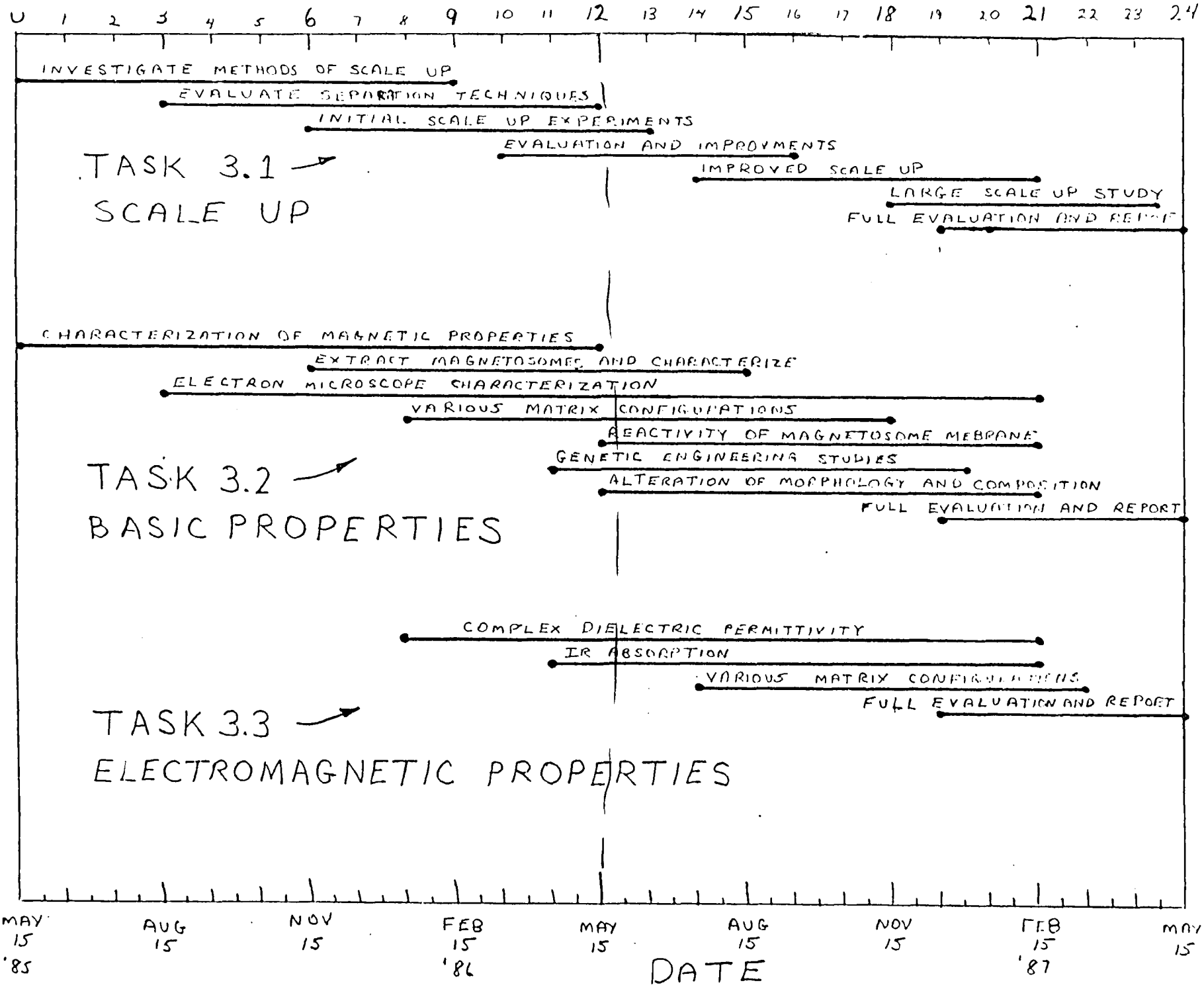


FIGURE 8



BUDGET SPENDING RATE


0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
INVESTIGATE METHODS OF SCALE UP
EVALUATE SEPARATION TECHNIQUES
INITIAL SCALE UP EXPERIMENTS
EVALUATION AND IMPROVMENTS
IMPROVED SCALE UP
LARGE SCALE UP STUDY
FULL EVALUATION AND REPORT
TASK 3.1
SCALE UP
CHARACTERIZATION OF MAGNETIC PROPERTIES
EXTRACT MAGNETOSOMES AND CHARACTERIZE
ELECTRON MICROSCOPE CHARACTERIZATION
VARIOUS MATRIX CONFIGURATIONS
REACTIVITY OF MAGNETOSOME MEBRANE
GENETIC ENGINEERING STUDIES
ALTERATION OF MORPHOLOGY AND COMPOSITION
FULL EVALUATION AND REPORT
TASK 3.2
BASIC PROPERTIES
COMPLEX DIELECTRIC PERMITTIVITY
IR ABSORPTION
VARIOUS MATRIX CONFIGURATIONS
FULL EVALUATION AND REPORT
TASK 3.3
ELECTROMAGNETIC PROPERTIES
MAY 15 '85
AUG 15
NOV 15
FEB 15 '86
MAY 15
AUG 15
NOV 15
FEB 15 '87
MAY 15
DATE